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THE VASCULAR ANATOMY OF THE SEEDLING OF *DIOON EDULE*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 119

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(WITH PLATES XXIII-XXIX)

This investigation was begun during the winter of 1906, the original purpose being to clear up the confusing statements in reference to the so-called girdling habit of the leaf trace. As the work progressed, its scope became extended, until finally it included all of those anatomical features of the seedling that have a bearing upon the relationship of the Cycadales to the Cycadofilicales and Filicales.

I wish to express my appreciation of the constant encouragement received at all times from Dr. JOHN M. COULTER, under whose direction the work was carried on; I also wish to thank Dr. C. J. CHAMBERLAIN for kindly furnishing the material.

Historical

The first work on *Dioon edule* was by METTENIUS (4), who compared it with *Cycas revoluta*, which was the special form investigated. The methods of those days (1861) did not permit tracing the various vascular bundles throughout their course. Sections were cut only here and there, and the large number of bundles presented only a very incomplete and vague idea to the investigator, resulting in complete misapprehension.

The picture of the leaf trace girdle as METTENIUS drew it is in the main as follows. A bundle in its course toward a leaf divides soon after leaving the central cylinder, the two branches in turn soon dividing. These branches and branchlets, in the main retaining their radially ascending direction, but running at various angles, anastomose with one another and with branches of neighboring bundles, and finally unite with bundles which girdle the vascular cylinder. This girdle lies closest to the ring at a point diametrically opposite the leaf base which its ends enter, each of which therefore traverses the cortex through an arc of about 90°, gradually separating farther from it and

finally entering the leaf base. There is such a girdle for every leaf, and every girdle must cut, on account of its course and location, every other like girdle in two places. Every girdle receives on its inner edge branches from the central vascular bundles which leave the vascular cylinder at various places, and sends out branches from its outer edge to other girdles. From this vague conception, which does not at all agree with the drawings of METTENIUS, the current textbook accounts have been drawn. These accounts, however, do not really interpret METTENIUS, but are as far from his interpretation as that was from the true situation.

In his description of the structure of the bundles METTENIUS was more fortunate, and made a very important contribution. He says that those bundles which are to leave the vascular cylinder are marked off by broad medullary rays and are more definitely bounded than those which continue in the cylinder; that on the inner edge they are provided with spiral vessels (protoxylem), while the others are provided with reticulated cells in the same relative position. At the region of the outward bend of the trace the vascular elements are grouped with the spiral elements on the inner or upper side, immediately bordering the reticulated elements beneath; and this structure the girdle retains in encompassing the stem. Before entering the leaf, however, a change in structure begins to occur, and is completed in the lower part of the petiole; after which the bundle remains unchanged up to the pinnae. The first indication of this change is the appearance of thin-walled cells in the vicinity of the spiral (protoxylem) elements, separating them from the reticulate elements. During the further course of the bundle the spiral (protoxylem) elements gradually move farther within, and the wood is now divided into two parts by the thin-walled cells; the inner part developing its elementary constituents centripetally, and the outer part centrifugally. Finally the spiral (protoxylem) elements are found in the outer part of the bundle, and the centrifugal part is still more reduced, while the centripetal part has reached its maximum development. This structure of the bundle is retained in its further course in the petiole until in the pinnae, where in *Dioon edule* the centrifugal wood is lost altogether. Such is a very brief statement of the description of the transition from centrifugal to centripetal xylem in the leaf traces, as

given by METTENIUS. It was the first correct statement of the facts, but their meaning was probably not understood until an interpretation of the situation was given in 1886 by BERTRAND and RENAULT (5).

The second paper upon the vascular anatomy of *Dioon* is that of MATTE (6), a brief description of the anatomy of two seedlings being given. In one of them, a very young seedling, the cotyledons were unequal, the larger having four vascular bundles, and the smaller having two small bundles, but with two other very small strands at the very base, one on each side of the other bundles. The bundles continue in a vertically descending course until where the foliar bundles, after being arranged in a circle, have been reduced to four large bundles, separated by medullary rays. The six cotyledonary strands turn now abruptly toward the three poles (protoxylem groups) of the root, converge, and unite two by two in front of them, effecting an entrance through the medullary rays separating the foliar bundles, and unite their phloem with the phloem of the stem. Their secondary xylem unites laterally with that of the stem, while the primary xylem seems to be in direct continuity with the tracheal poles (protoxylem groups) of the root. The centripetal xylem disappears in the passage across the medullary rays.

In the description of the older seedling MATTE found in each cotyledon four bundles of equal size. Their course and method of union is comparable with that found in the other seedling, and they still unite two by two in converging toward the poles (protoxylem groups), but these poles are four in number.

MATTE touches also upon the girdling habit, ascribing it to an intercalary growth produced under the influence of the development of new interior leaves. The vascular strands of the youngest leaves pursue a vertical course, but those of the older ones an oblique course, a comparison of leaves of different ages showing that this departure from a direct course is due to intercalary growth.

Methods

Embryos were removed from mature seeds and killed in chromo-acetic acid, imbedded in paraffin according to the usual methods, cut with a rotary Minot microtome, and mounted in series, much care being taken that no sections were lost or misplaced. When only the

location or outline of the bundle was wanted, the cross-sections were cut 20 to 30 μ thick; in other cases they were cut 10 μ thick. Longitudinal sections were cut at right angles to the inner surfaces of the cotyledons, as well as parallel to that plane; these were cut 10 μ thick. Staining was done on the slide with safranin and Delafield's hematoxylin, or safranin and anilin blue. Other stains were tried, but these two combinations gave by far the best results. A few specimens were killed by the general picro-mercuric-chlorid method, but no advantage was gained.

Where seedlings were required, the hard coats of the seeds were cut partly open at the micropylar end, and the seeds placed on one side, partially pressed into moist earth, in a flower pot, and put in a warm moist place in the greenhouse and kept well watered. A good many seeds had been planted three years before and had grown to considerable size, some of them having a stem 100^{mm} in length and 25^{mm} in diameter, and bearing several leaves. These were treated about as the embryos, except that all the specimens were cut into lengths of 15 to 20^{mm}. Of course the greater the size of the plant the more slowly the processes of killing, washing, embedding, etc., were conducted. Especial care had to be taken in imbedding the larger specimens, the best results being obtained when they were carried through the process of penetration with paraffin for a whole month. After this time they could be cut with an ordinary Minot rotary microtome with perfect ease, and the sections could be held in complete ribbons. After this precaution, ribbons were obtained from specimens 26^{mm} in diameter and cut 10 μ thick. Staining was done as before with safranin and Delafield's hematoxylin, or safranin and anilin blue, the former combination giving the most satisfactory results. An enormous amount of labor is necessary and also much care to keep sections of entire plants in serial order, both in cross and longitudinal sectioning, but it is the only satisfactory method.

In order to obtain the details or to follow up the leaf trace courses, every section was examined, from the first to the last, and camera drawings made of every second or third section. These were carefully numbered and kept in series and later compared, and reconstructions were based upon them.

Observations

THE EMBRYO

EXTERNAL STRUCTURE.—In the mature seed the cylindrical embryo is fully two-thirds as long as the endosperm, averaging 20^{mm} in length and about 4^{mm} in diameter. The hypocotyl (*fig. 1*) is comparatively very short, being about 5^{mm} long, ends abruptly (very often is even concave), and in the center is still attached the slender, very much twisted and coiled suspensor (*s*). The cotyledons are free for the upper four-fifths of their length, the lower fifth forming a tubular sheath (*sh*) inclosing the leaf primordia (*figs. 2, 3*). One of the cotyledons is slightly larger than the other, and is inserted a little lower on the axis, slightly enfolding the smaller one. There are generally two leaf primordia, but sometimes three. The broad base of the outer or older one (*L*₁) embraces the inner and next younger one (*L*₂), which in turn often embraces a third (*L*₃); and finally beneath them all is the stem tip (*st*). On the oldest leaf primordia are all the rudiments of the pinnae (*pn*) of the future leaf quite well advanced; while no indication of them can be detected on the younger primordia.

To understand certain features to be described later, it is necessary to note the arrangement of the earlier leaves. An older plant (*fig. 7*) may be used as an illustration, and both scales and foliage leaves will be spoken of as leaves and numbered from 1 to 9 according to age. The cotyledons (*col*) are apparently exactly opposite; 1 and 2 are approximately opposite, but close observation shows that they are not exactly so; also 1 and 2 are approximately at right angles to the cotyledons (also *fig. 2*). The sheathing leaf base of 1 is a little longer and more slender on the side toward which the spiral turns, and folds around the inner leaf or scale a little farther than it does on the other side. Leaf 3 lacks still more of being opposite 2; also the corresponding edge of the sheathing base of 2 is more slender and folds over 3 more than its fellow. Leaf 4 lacks still more of being opposite 3, and again the sheathing base is more slender and folds around the next inner leaves more on that side toward which the spiral turns. In the rest of the series the spiral is uniform, and the overlapping more conspicuous.

INTERNAL STRUCTURE.—The bulk of the embryo is of course

parenchymatous, and the general structure is shown in *figs. 2-6, 8, 9, 29-35*.

The epidermis (ep) consists of large, regular, isodiametric cells with large nuclei. That of the cotyledons is abundantly supplied with stomata on the exterior surface of the tubular part and a small portion of the lobes. No stomata occur on the part of the cotyledons remaining in the endosperm, on the interior surface of the cotyledons, or on the primordia. The epidermis of the petioles and bases of the leaf primordia is covered densely with long, unicellular hairs, with well-defined nuclei (*figs. 28, 29*).

The ground tissue is composed of long, regular, prismatic cells, much longer than wide in the cotyledons, but shorter and more isodiametric in the stem proper (*figs. 3, 8*). At the lower extremity of the embryo the cells lose their nuclei, become filled with a dense material, and form a hard capping tissue (*cp, figs. 3, 28, 29*). Distributed irregularly in all parts of the ground tissue are many cells, cavities, and canals filled with mucilage (*m, fig. 8*; black spots in *figs. 28-35*). The cavities are found most abundantly in the cotyledons and are formed by the disorganization of several neighboring cells. The canals, which are formed by the disorganization of the cells end to end, forming tubes of limited length, are most abundant in the petioles of both cotyledons and leaves.

The vascular cylinder is very short, the length being much less than the diameter, and hence it is usually called the vascular plate (*vp*). It is squarish, one diagonal diameter being approximately at right angles to the inner faces of the cotyledons, and the other parallel with them. The xylem (*figs. 32, 33, x*), consisting at this stage probably of protoxylem only, is surrounded by a zone of phloem (*ph*). The xylem is compact and well developed where it borders the phloem, but toward the center it is gradually mixed more and more with pith cells, till in the center the xylem elements lie scattered among the pith cells, these scattered cells being very short and irregular. This condition varies in different specimens, in some the xylem being quite compact to the center, while in others it is entirely wanting in this region (*fig. 32*).

The protoxylem groups.—At each corner of the squarish vascular plate there is a group of protoxylem elements (*px*), which in cross-

section is irregularly oval. These four groups extend downward for a short distance, where they form the protoxylem of the primary root (*a*, *figs.* 4, 5, 8).

The potential vascular tissue.—Above the vascular plate there is a conical or dome-shaped mass of tissue terminating in the growing point of the stem, sharply marked off from cortex and pith, and consisting of long, irregular cells with dense protoplasm and prominent nuclei. The frequent occurrence of mitotic figures shows it to be the most active meristematic region of the stem, being the tissue in which the vascular strands are developed, and therefore the procambium (*pc*, *figs.* 8, 34, 35).

The pith in the vascular plate contains scattered and short xylem elements (*x*, *fig.* 8), as stated above. Above the plate it is conical and terminates in a tier of cells against the epidermis (*pt*, *fig.* 8); in this region the cells are thin-walled and very irregular. In all the pith mucilage cells and cavities are abundant.

The vascular strands.—All the vascular bundles are collateral, except in the upper part of the cotyledons, where they may be regarded as concentric. In the leaf strands the collateral bundle is surrounded by a sheath, which is not well-defined. In the younger strands the few elements are protoxylem, and new elements are added centrifugally (endarch), centripetally (exarch), or in all directions (mesarch). The bulk of the bundle is as yet procambial tissue (compare *figs.* 10-15 and 16-21 with *figs.* 22-25). In the older strands it is difficult, if not impossible, to determine where protoxylem ends and metaxylem begins.

The vascular strands of the cotyledons (*figs.* 4-6).—From each of the four protoxylem groups in the vascular plate (*px*) a strand runs outward for a short distance and then branches, the branches separating at wide angles and continuing outwardly in a horizontal plane until well under the bases of the cotyledons, where they turn abruptly upward into the cotyledons, thus giving four strands to each cotyledon, and in such a way that each of the opposite protoxylem groups on the diagonal perpendicular to the inner faces of the cotyledons gives rise to the two inner strands of each of the cotyledons; while each of the other two protoxylem groups gives rise to the outer strands of the cotyledons in opposite edges, that is, one branch goes into the

edge of the cotyledon on one side, and the other branch from the same protoxylem group into the corresponding edge of the other cotyledon. This may be stated in another way. In tracing downward, the four strands of each of the petioles of the cotyledons may be said to join two by two. Just before reaching the central cylinder the inner strands of each fuse, and the outer strands of the one fuse with the outer strands of the other, the four strands thus formed giving rise to the four protoxylem groups. This is shown semi-diagrammatically in *figs. 4-6*. Tracing these strands farther upward, they are found to branch once more, so that in the upper part of each cotyledon there may be as many as eight strands; but before reaching the tips of the cotyledons they reunite into one concentric bundle (*fig. 15*), which abuts immediately against the epidermis, thus coming into very close contact with the gametophyte. At this place the tissue of the gametophyte is so closely attached to that of the cotyledons that it is difficult to separate them.

The vascular strands of the leaf primordia.—For each leaf or leaf primordium four strands leave the vascular cylinder or vascular plate, at points not definitely located, but quite well distributed, and generally in such a way that approximately one strand for each leaf or primordium leaves on each side of the squarish central vascular cylinder; also those strands belonging to the first leaves have their origin either in the neighborhood of or in the protoxylem groups of the plate. Two strands leave the cylinder approximately on the same side as that on which the leaf for which they are destined is located, and run more or less directly through the cortex into the ventral part of the petiole without further branching; while the other two strands leave the central cylinder approximately on the opposite side and describe a curve around it (the one in one direction and the other in the opposite direction) through the cortex, through the sheathing leaf base, and finally into the dorsal or adaxial part of the petiole, where they branch and rebranch (*figs. 4-6*). It should be emphasized that the point of origin is not at all definite, and that any particular girdle does not describe an arc of any definite extent, but that the length of the arc depends upon the place of origin of the girdle and the position of the leaf to which it belongs.

It has been said that that edge of the leaf base toward which the

spiral turns is more slender and folds over the next inner leaf more than does its mate (*fig. 7*). It will be observed that the girdle which is destined for that side of the leaf generally describes a longer curve through the cortex than the one destined for the other side. The phenomenon of girdling will become clearer when illustrated by a specific case. In *fig. 6*, taken from a young seedling, L_1 , L_2 , L_3 , and L_4 represent the first, second, third, and fourth leaves or primordia; L_1^1 , L_1^2 , L_1^3 , L_1^4 , the four strands of the first leaf; L_2^1 , L_2^2 , L_2^3 , L_2^4 , the strands of the second primordium. L_1^1 is the longest girdle of the first leaf and has its origin at the protoxylem group px^1 , on the side directly opposite the leaf to which it belongs, ascends for a short distance, then turns outwardly into the cortex and describes a wide horizontal curve, enters the longer and more slender edge of the leaf base, and ascends in the petiole in the dorsal left-hand portion, branching repeatedly in its further course. Trace L_1^4 leaves the central cylinder near the protoxylem group px^4 , ascends a short distance, runs upwardly and outwardly into the cortex, describes a horizontal curve in the opposite direction, enters that side of the leaf having the shorter sheathing base, and ascends in the dorsal or adaxial portion of the petiole, where it branches repeatedly in its further upward course. From this it will be seen that the leaf trace L_1^1 pursues a longer course than trace L_1^4 . Trace L_1^2 has its origin near the protoxylem group px^3 , to the left, ascends for a short distance, runs out into the cortex, makes a very slight horizontal curve (almost direct) into the leaf base, running outwardly, and then ascends on the ventral or abaxial left-hand portion of the petiole without further branching. Trace L_1^3 has its origin near the protoxylem group px^4 , ascends for a short distance, runs outwardly (gradually ascending) with a slight curve into the leaf base, and finally ascends in the ventral or abaxial right-hand portion of the petiole without further branching. It will be seen that in the case of the two inner strands L_1^3 makes a girdle of about 90° , while trace L_1^2 is approximately direct.

About the same condition occurs in the traces of the second leaf. Trace L_2^1 has its origin to the left and near the protoxylem group px^3 , ascends vertically for a short distance (but farther than the strands of L_1), runs a short distance outwardly into the cortex and then makes a wide horizontal sweep, enters the leaf base on that side

which ensheaths the younger primordium with its more slender edge, ascends in the petioles on the inner or dorsal portion, and branches repeatedly. Trace L_1 has its origin near and to the right of the protoxylem group px^2 , describes a girdle of approximately the same magnitude as girdle L_1^4 , and runs into the base of the leaf on the right hand. Traces L_2^2 and L_2^3 have their origin respectively on the right and left of the protoxylem group px^1 (quite close to it), and after ascending for a short distance run directly into the outer or ventral portion of the leaf, where they ascend without further branching.

These facts seem to indicate that there is some relationship between the protoxylem groups of the vascular plate and the origin of the trace of the first leaves. The traces of the third leaf (L_3) are followed with some difficulty, but the same condition noted for the first and second leaves is clear. When a fourth leaf is far enough advanced, exactly the same conditions are also presented. The girdling habit of the very young strands is already marked out; but their origin appears no longer to be restricted to the neighborhood of the protoxylem groups, but may occur anywhere in the plate, from which they now ascend vertically for a longer distance before turning out into the cortex. It is very difficult, however, to follow the younger strands while they are still in the potential vascular tissue, since no xylem elements have as yet been formed; but in their passage through the cortex into the primordium their path may be made out clearly, and it shows that the girdle is already established at this stage. Even in the absence of xylem elements, the bundle is clearly marked off by the character and arrangement of its cells (*figs. 26, 27*).

Although the specific case described represents the general state of affairs, many variations are found in the place of origin of the traces. Of the many specimens examined probably no two traces were found to be exactly alike in this respect; also anastomoses between adjoining traces were found here and there. The strands thus ascending vertically for a short distance into the procambial tissue above the vascular plate are the first to assume specific characters. Although the young cylinder is made up of separate traces, it must be observed that these are in the procambial tissue, which is very different from that which surrounds the procambium.

The transition from endarch to exarch xylem.—When a cotyle-

donary strand leaves the vascular cylinder it is endarch (*fig. 10*). As it passes upward and outward, the protoxylem elements recede from the endarch position and are buried more and more in metaxylem, that is, centripetal wood has appeared. When well up in the tubular portion of the petioles of the cotyledons, the protoxylem is surrounded equally on all sides by metaxylem, the xylem being typically mesarch (*figs. 11-13*). From this point on the protoxylem approaches more and more an exarch position, but the xylem does not become completely exarch. Before the end of the bundle has been reached it has become quite concentric, and it becomes very difficult to determine which element was the first to appear (*fig. 15*). Where the bundle leaves the central cylinder the total xylem elements so far as developed are at the innermost part of the bundle, and as the bundle is traced upward they recede from that position and occupy one farther inward, until in the upper extremity of the cotyledon they occupy a position central to the whole bundle. Thus the transposition of the total xylem holds the same relation to the procambium as the protoxylem holds to the metaxylem (*fig. 9, a*).

The leaf traces also when leaving the vascular cylinder are endarch (*fig. 16*), and in passing outward and upward the protoxylem elements recede from the inner edge and are buried deeper and deeper in the metaxylem; and well up in the leaf bases the xylem has become mesarch (*figs. 18, 19*). Afterward the protoxylem approaches more and more an exarch position, until at the transition between petiole and leaf base the xylem has become completely exarch, with the protoxylem lying immediately against the procambium (*figs. 20, 21*). There is at this stage no centrifugal wood above this point; and there is no secondary wood anywhere. The transition may be said to occur, therefore, between the central cylinder and the leaf base, from which point upward the strands are all exarch. It must be taken into consideration that none of the leaves are as yet fully developed, only the first leaf showing plainly the different regions (*fig. 3*). *Fig. 9, a* represents the situation diagrammatically.

It will be observed that only a very small part of the procambium has been developed into xylem tissue in the whole length of the petioles (*figs. 16, 17*). Cross-sections of foliar strands at a low region show, besides the protoxylem and centrifugal metaxylem elements, a pro-

cambium stretching more than half-way across the bundle, the boundary being marked by a thicker-walled tissue, the protophloem. Higher up in the bundle the centrifugal procambium decreases proportionally as the protoxylem moves outward. *Figs. 4, a and 21* show the situation more clearly; both are taken from very young primordia, the latter much younger and higher up than the former. In *fig. 21* the xylem elements are approximately in the center of the bundle, but there is still considerable procambium between them and the protophloem. The cambium appears to be developed later, just inside the protophloem, and develops tissue actively toward the phloem side only.

THE SEEDLING

EXTERNAL STRUCTURE.—When the seed of *Dioon* germinates, the hypocotyl pushes through the micropylar end of the seed, where there is at this time an area in the testa, about the diameter of the embryo, which softens readily and is easily penetrated by water. If left to itself, this process takes from a week to a month, and sometimes longer; but if the area referred to is cut away, the hypocotyl pushes through in a few days. During germination the whole of the hypocotyl and the lower part of the cotyledons lengthen.

As soon as the hypocotyl reaches the soil a tap root is sent deep into the ground, and before any leaves appear the root may have penetrated the soil 8 to 12^{cm}, and the diameter of the hypocotyl may have increased to about 1^{cm}. After a long period (which in the greenhouse was about four weeks in case the micropylar end was cut away, but many months if it was not) the first leaf appears between the cleft of the cotyledons, in direct continuation of the axis. As the natural position of the seed during germination is horizontal, and the hypocotyl bends down approximately at right angles, the leaf pushes forth where the cotyledons make a sharp bend. The cotyledons remain in the seed, and in seedlings three or four years old the seed may be seen still attached to the plant. At this time also lateral roots appear in four rows along the primary root, corresponding to the four protoxylem groups.

A second leaf does not appear until perhaps a year later, and about another year elapses before a third appears. A plant of this

age has developed about nine or ten leaf primordia, but only about one-third have developed into leaves.

INTERNAL STRUCTURE.—During germination, in connection with the development of the primary root, not all of the tissue of the tip of the plantlet resumes growth, but only the plerome and a limited portion of the periblem or cortex surrounding it becomes meristematic and pushes through the hard and caplike tissue at the tip of the embryo. The cortex which does not resume growth, as well as the caplike tissue, frays off (*fig. 9, jr*). Also, as growth progresses, the outer layers of the newly formed root, some distance back of the root tip, keeps on fraying off; and underneath a phellogen soon appears and a layer of cork several cells thick is formed. Along each of the four protoxylem strands of the root, continuous from the vascular plate, lateral roots have their origin at definite intervals, being arranged in four rows. Certain cells among and near the protoxylem elements become meristematic, and form the tip of the lateral root, which pushes through the cortex.

The vascular cylinder increases in dimensions uniformly with the growth of the seedling as a whole. New bundles are inserted as new leaf primordia appear, and gradually fill in more and more the vacant spaces between the original strands, so that immediately above the original plate the vascular cylinder is quite compact, while beyond this it continues to be represented by separated strands in the potential vascular tissue.

The vascular strands of the cotyledons.—Not much need be added to the statements in reference to the cotyledonary strands. The transition from endarch to exarch xylem is very much more gradual and the mesarch stage is located relatively much farther up. This is due to the fact that most of the growth of the cotyledon in length occurs at its lower extremity. No secondary wood is developed in cotyledons. The phloem also has increased in bulk, but mature sieve tubes are never developed.

The leaves and scales.—As said above, not all the primordia develop into leaves, most of them remaining abortive and forming scales. Although about one-third of the primordia develop leaves, it does not follow that every third primordium becomes a leaf in regular succession. It sometimes happens that two leaves are developed from

consecutive primordia. Evidences indicate that a scale is not predetermined, but remains abortive through some variable cause. The primordia, whether developing scales or leaves, show the same structure in every particular.

The leaf traces.—The course of the leaf traces in the seedling are the same in general plan as those described for the embryo, but the girdling in the older leaves and scales is much more marked. The internal growth and the appearance of new organs has crowded the older parts farther and farther outward. The circumference of the cortex has increased materially, and also the length of the vascular strands running through it. In the older scales and fully developed leaves these are all of about the same extent, and almost horizontal; but from these, through the younger leaves and scales to the youngest primordia, the sweep of the girdle diminishes; but the girdles are already established in the very youngest of the primordia (*figs. 34, 35, ug.*)

Although in the strands of the youngest leaf primordia no xylem elements are present, the courses of the bundles may be made out readily because of the arrangement, the staining qualities, and shape of the cells, which have denser protoplasm and larger nuclei, and are longer than the adjacent cells (*figs. 26, 27*). The strands of the very youngest primordia which have their origin on the opposite side of the central cylinder show the girdling habit in the same manner as those of the older leaves, but on a smaller scale. The girdle does not always take the horizontal direction, but may be more oblique at the beginning (*figs. 4, 5, 34, 35*). *Fig. 35* shows this clearly, in which *pc* is the procambium and *ug* the girdle of the youngest primordium. So long as such a strand is outside of the procambium it can be followed easily, but is lost after it has entered it.

In the oldest plant examined (three or four years old), the first, second, third, and fourth leaves displayed exactly the same condition as was shown in the young leaves of the embryo and seedling. But in the older leaves, outside of these, it is impossible to determine whether the described order is retained, because of the difficulty in following up strands of such size; but it is certain that anastomoses are more frequent, due to the close proximity of crossing bundles.

The transition of the xylem.—The strands of the older leaves have

a larger development of metaxylem; also secondary wood may have developed in the lower extremities, so that the transition from endarch to exarch has become more prominent and can be made out with greater clearness. The secondary wood accompanies the bundle as yet only for a short distance, and ceases long before the transition from endarch to exarch is complete. As the secondary xylem and centrifugal metaxylem diminish, the centripetal xylem (which of course is all primary) increases in bulk (*fig. 9, a*). In a plant three years old no other secondary wood was present (*figs. 22, 23*). Even in the oldest leaves of quite old plants the secondary wood, which at the origin of the strand is quite massive, decreases very rapidly, and in the petiole just above the leaf base has thinned out to a few elements (*fig. 24*), remaining quite uniform to the rachis, where it disappears still more; while in the pinna no secondary wood whatever is present, all the xylem being primary and centripetal. Although in the transition region the secondary wood diminishes in the same ratio in which the primary wood increases, it must be noticed that the centrifugal wood is not restricted to the secondary wood alone, as was shown in the younger bundles of the embryo, where the transition is clearly carried out in the protoxylem and metaxylem alone. Thus in the seedlings the transition from centrifugal to centripetal wood is carried on after the appearance of secondary wood, and is completed in the primary wood.

In the older strands where secondary wood has been developed, a considerable amount of the centrifugal wood therefore is metaxylem. This is shown by the amount of procambium that has been developed into centrifugal xylem; as may be seen by comparing the younger strands in *figs. 21, 4, a*, for example, where there is a certain amount of centrifugal procambium, the amount depending upon the distance from the point of egress from the central cylinder, with the older strands in *fig. 24*. Sometimes all of the centrifugal procambium has become xylem; more often, however, patches of procambium or isolated cells of it are never lignified and retain their nuclei, and are then referred to as the thin-walled cells. These thin-walled cells do not necessarily lie against the secondary wood, though they most often do, and become most evident in the upper extremities of the transition. A series of cross-

sections of a maturer leaf of a seedling clearly shows the transition from endarch, through mesarch, to exarch, and shows that it is quite independent of secondary wood. The transition begins at the point where the strand leaves the central cylinder, and in the seedling progresses uniformly and is completed in the petiole; but in the old plant the larger proportion of the transition is completed in the lower part of the petiole, where only a few strands of secondary xylem remain and continue uniform until the bundles enter the pinna, when the transition is completed (*fig. 25*).

The cause of girdling.—No particular cause has been assigned for this phenomenon. If such a leaf primordium as is represented in *figs. 3, L₂, and 8, L*, be selected, it is possible by careful staining to detect four strands which are ultimately developed into vascular bundles. The outer (abaxial) ones pursue a more or less direct course, but in following them from the stem up, their course at first, after leaving the procambium, is vertical; but in the base of the primordium they turn inward to a considerable extent (*fig. 3, L₂*). The two inner or adaxial strands after leaving the procambial cone pursue quite a vertical course, but on reaching a region at the level of the base of the primordium, they begin to turn toward it, one on each side, in some cases ascending rather obliquely, but generally horizontal from the start. *Figs. 34, 35, 4g¹* shows the girdling of quite a young primordium, the youngest one in that specimen. It is plain, therefore, that the girdle is established very early in the development of the leaf to which it belongs.

Tracheae.—In examining older plants, the oldest one being three or four years old, a singular phenomenon is noticed. Between the various bundles of the stem, which at this time do not yet make a complete ring, vertical connections are found, consisting of irregular, reticulated elements, branching and anastomosing, but forming continuous vessels. In the upper part of the plant, where they are developing, long and narrow cells are found winding and crowding through between the parenchymatous cells. A little farther down in older parts of the stem, these cells are found to be multinucleate, with only here and there an ill-defined cross-wall. A little farther down still the nuclei disappear, and soon lignification appears, developing finally into well-defined reticulated tracheae. These wind in a very tortuous

way vertically through the parenchymatous cells from one bundle to another. They are in all senses true reticulated vessels, without cross-walls, establishing a connection between the bundles of the vascular system.

Discussion

In the embryo of *Dioon edule* the vascular cylinder is a protostele, which in some specimens contains a solid xylem mass. From this solid cylinder all gradations are found to the siphonostele. The cells of the pith are actively meristematic, as shown by the mitotic figures, and often in older specimens xylem elements are found in the central part at the level of the vascular plate. The xylem cylinder also continually increases in size, new elements being added to it constantly between bundles already existing, as well as by the cambium.

It should be emphasized that the vascular cylinder in the embryo and seedling does not consist of the short xylem cylinder only, but is continuous in a tissue, very different from the cortex outside and the pith within, which gives rise to strands of procambial tissue running into the leaf primordia. The pith is also a well-defined tissue from the vascular plate to the stem tip, and nowhere suggests that it arises from an intrusion of the cortex through the leaf gaps. An inspection of *fig. 8* shows that it has its beginning at the very tip of the growing point, where it is seen to consist of a single row of cells or a tier of a few cells, gradually expanding as the stem grows into the large pith found in the older part.

The transition of the protoxylem from the endarch to the exarch position was first described by METTENIUS (4), who also suggested the descriptive terms centripetal and centrifugal xylem. The situation was interpreted by BERTRAND and RENAULT (5), who also established that the centripetal wood is in the same relative position throughout its whole length; that it increases in bulk toward its upper end; and that the centrifugal elements are reduced more and more. They say that the centripetal wood is intercalated between the pole and the outer face. It is regarded as the primary wood (developed from the procambium), while the centrifugal wood is regarded as secondary (developed from the cambium).

The present investigation shows that this statement needs modification. While all centripetal wood is primary, all primary wood is

not centripetal, as one would interpret from the treatise cited. It has been shown that the transition from endarch to exarch is carried through in the metaxylem, both centripetal and centrifugal wood occurring long before secondary wood is developed. It is very hard to tell where protoxylem ends and metaxylem begins, and where metaxylem ends and secondary wood begins. The separation of metaxylem from secondary wood by means of the thin-walled parenchymatous cells is not a safe guide in the region of transition, and only becomes well marked above this region, where the separation of secondary from primary wood is well marked by the thin-walled parenchymatous cells, as noted by many authors. The metaxylem above this point gets to be relatively very bulky, while the secondary wood is represented merely by a few elements. These were the few pitted cells which presented to VON MOHL (1) a situation without a counterpart, now known to be the herald of the secondary wood which has gradually crept up into the petiole, a transformation begun in its early ancestry, according to SCOTT, the "new wood" driving out "the old," the former being the only wood present in the higher gymnosperms and angiosperms.

MATTE (6) argues in very much the same way as does METTENIUS, an argument which would hold good if the protoxylem and a large part of the metaxylem were left out of account. MATTE says that the bundles of the cotyledons have centripetal wood throughout, centrifugal wood only below the upper region of the petioles, and centripetal and centrifugal wood equally well developed at the bases of the petioles. In the present investigation it has been pointed out that there is no centripetal wood at first, and that it gradually increases; while the centrifugal wood diminishes in bulk to the upper extremities, where it is less than the centripetal but does not disappear entirely.

MATTE further says that what has been said of the cotyledonary traces applies equally well to the foliar traces, except that there is no trace of centrifugal xylem in the youngest leaves. It can be shown that as soon as there are enough xylem elements to show the direction of development the centrifugal wood is present, but gradually disappears, and the centripetal wood increases in the same ratio, until in the upper extremities there is only centripetal wood. This also agrees with BERTRAND and RENAULT, except that their statement that centripe-

tal wood is primary and centrifugal wood secondary does not hold true, a mistake apparently shared by MATTE. It has been shown that protoxylem and metaxylem may have both centripetal and centrifugal elements. This is very well seen in cross-sections at a low level of a young strand, where only a few xylem elements are developed. In such a section the protoxylem lies against the inner edge of the bundle, and the procambium can be seen to occupy considerably more than half of the bundle; the boundary of the procambium and the protophloem is distinctly recognizable, the protophloem forming only about one-third of the bundle. In the upper extremities of the petiole, also, there may be seen at an early stage a considerable amount of procambium outside the protoxylem; as these usually fail to develop xylem, thin-walled cells occur between the primary and secondary wood in the upper extremities (*fig. 21*).

The girdling habit was first noticed and described by KARSTEN (2) in *Zamia muricata*, without, however, giving the definite number of traces. LESTIBOUDOIS (3) adds nothing new except that the traces branch and anastomose. METTENIUS (4) next misinterpreted the situation, as described in the historical introduction (p. 357).

In the embryo and young seedling, at least, the leaf traces pursue definite and well-defined courses and constitute a definite system. Four traces are invariably found to leave the vascular cylinder for each leaf. A few anastomoses occur here and there, but these are always reducible to four strands. On two occasions only five strands were found in one of the cotyledons. In the older seedlings anastomoses are more abundant, but so far as observed these can be reduced to the system found in the young seedling.

When KARSTEN (2) described the girdling habit, he suggested a cause for it in saying that the bundles are formed very early in the young leaf, and that the originally narrow curves are later crowded far out by subsequent growth and the appearance of new organs. METTENIUS (4) also gives a reason for girdling as follows: "In the developmental stage the traces of the youngest leaves lie in the region of the vegetative point, and at first ascend in an almost perpendicular direction, but during the further growth assume gradually an almost horizontal position, and with subsequent growth are lengthened and

the expanse is increased." MATTE (6) assigns almost exactly the same cause.

A definite cause for the girdling cannot be given at present, for it seems to be deeper seated than at first suspected. The even distribution of the four strands of each leaf in the vascular cylinder appears to be the dominating factor. When the primordium appears on the stem tip, its distance from the potential vascular cylinder is very short, and the same conditions that determine cell division in the development of the leaf, cause the differentiation of cells along certain paths that run from definite points in the procambium to definite places into the developing leaf. After the traces have been started, they continue to develop with the further growth of all the tissues; new organs appear and intercalary growth continues; thus the strands are lengthened more and more and their curves are widened to keep pace with the ever increasing growth of the plant.

Summary

1. The vascular cylinder of the embryo is a protostele, but becomes a siphonostele in the seedling. It is very short and squarish in cross-section, one of the diagonals of the section being at right angles to the inner faces of the cotyledons, and the other parallel with them. Near each of the four corners is a group of protoxylem cells, the long diameter of whose section extends along the diagonal.

2. The four protoxylem groups extend downward to form the protoxylem of the root.

3. From each protoxylem group a foliar strand runs outward for a short distance and then branches, the branches separating at a wide angle and continuing outward until under the cotyledon, when they ascend it; the result is four strands for each cotyledon. The two protoxylem groups on the diagonal perpendicular to the inner faces of the cotyledons give rise to the two inner strands of each cotyledon; while the two protoxylem groups on the diagonal parallel with the inner faces of the cotyledons give rise to the two outer strands of the cotyledons, that is, one branch runs into the outer edge of the one and the other branch (from the same group) runs into the edge of the other cotyledon (opposite the first).

4. For each leaf or scale four strands leave the vascular cylinder

at points not definitely located but well-distributed; two strands of each organ leave the cylinder approximately on the same side as the leaf for which they are destined, and run more or less directly through the cortex into the central or abaxial part of the petiole without branching; while the other two strands of each organ leave the cylinder approximately on the opposite side and describe a wide curve around it, the one in one direction and the other in the other, and finally ascend in the dorsal or adaxial part of the petiole, branching repeatedly. The girdle on the side toward which the spiral may be said to turn is generally the longer one.

5. When the cotyledonary vascular strands leave the vascular cylinder they are endarch, gradually become mesarch in their upward course, and finally approach the exarch condition.

6. The foliar vascular strands also are endarch at their separation from the vascular cylinder, and in their upward course become mesarch and finally exarch. In the very young leaf this transition extends through the whole base and petiole uniformly; but in the adult leaf it is comparatively rapid through the base up to the lower part of the petiole, where only a few centrifugal elements remain, and which remain uniform until in the rachis, where the transition is completed, so that in the pinna only centripetal xylem is left.

7. In the lower stretches of the foliar strands a considerable amount of the centrifugal wood is primary xylem.

8. In the foliar strands of the embryo and seedling, the xylem tissue, so far as developed, from below upward passes from an inner to a central position in reference to the whole bundle.

9. The girdle is established very early, and is horizontal from the beginning.

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EXPLANATION OF PLATES XXIII-XXIX

PLATE XXIII

FIG. 1.—Embryo in mature seed: *cot*, cotyledon; *sh*, tubular sheath of cotyledons; *st*, stem proper; *r*, hypocotyl; *s*, suspensor.

FIG. 2.—Cross-section of embryo just above stem tip: *cot*, tubular part of cotyledons; *L*₁, first leaf; *L*₁¹, *L*₁², *L*₁³, *L*₁⁴, vascular strands of first leaf; *L*₂, second leaf; *L*₂¹, *L*₂², *L*₂³, *L*₂⁴, vascular strands of second leaf; *L*₃, third leaf.

FIG. 3.—Median longitudinal section of lower part of embryo, parallel with inner faces of cotyledons: *cot*, cotyledon; *L*₁, *L*₂, first and second leaves; *pn*, pinna; *ls*, foliar strand; *st*, stem tip; *p*, pith; *cs*, cotyledonary strand; *vp*, vascular plate; *pl*, pterome; *cp*, caplike tissue; *sp*, suspensor.

FIG. 4.—Semi-diagrammatic reconstruction of part of vascular system of embryo; *cot*, cotyledon; *tb*, tubular part of cotyledons; *cs*, cotyledonary strands; *L*₁¹—*L*₁⁴, foliar strands of first leaf; *L*₂¹—*L*₂⁴, foliar strands of second leaf; *vp*, vascular plate; *a*, protoxylem elements continuing downward into the hypocotyl.

FIG. 4a.—Cross-section of a vascular bundle in upper extremity of young leaf; *x*, xylem elements; *a*, cells losing their contents during lignification; *pc*, procambium; *pph*, protophloem; *b*, line showing boundary between protoxylem and protophloem.

PLATE XXIV

FIG. 5.—Semi-diagrammatic reconstruction of part of vascular system of embryo, to show especially the girdling: *L*₁, *L*₂, traces of first and second leaves; *vp*, vascular plate; *px*, protoxylem groups; *a*, xylem elements continuing down from protoxylem groups to form the protoxylem of the primary root.

FIG. 6.—Diagram giving bird's-eye view of vascular system, to show origin and girdling of foliar strands: *cot*, tubular part of cotyledons; *cs*, cotyledonary traces, one group for each cotyledon; *L*₁, *L*₂, *L*₃, *L*₄, first, second, third, and fourth leaves; *L*₁¹, *L*₁², *L*₁³, *L*₁⁴, traces of first leaf; *L*₂¹, *L*₂², *L*₂³, *L*₂⁴, traces of second leaf; *px*, protoxylem groups.

FIG. 7.—Outline of cross-section just above stem tip of seedling three or four years old, to show phyllotaxy; *cot*, cotyledons; 1-8, leaves in order of age.

FIG. 7a (1).—Stem tip from germinating embryo with leaf primordium (*L*) developing on the side.

FIG. 7a (2).—Stem tip from rapidly growing seedling, showing growing point (*st*) in the middle and a leaf primordium (*L*) on each side; this section showed numerous spindles in growing point and primordia.

PLATE XXV

FIG. 8.—Details of central region of embryo in median longitudinal section: *L*, youngest leaf; *st*, stem tip; *ep*, epidermis; *pt*, pith terminating in stem tip; *pc*, procambium; *vp*, vascular plate; *ph*, phloem; *a*, continuation of protoxylem into the root; *p*, pith; *pl*, pterome; *m*, mucilage cells.

FIG. 9.—Median longitudinal section of lower part of germinating embryo, showing lengthening of pterome, development of primary root, and fraying-off of epidermis and outer part of cortex (*f*).

FIG. 9a.—Diagram to show transition of protoxylem in the metaxylem, and transition of primary xylem from an inner to a central position in a young bundle: *aa'*, inner, and *gg'*, outer limits of bundle; shaded portion (*ab*, *cd*), xylem tissue, *ad*, protoxylem; unshaded portion in *aa'* (*cf*), procambium; *cf* (*gg'*), protophloem; dotted line *cj*, boundary between procambium and protophloem.

PLATE XXVI

FIGS. 10-15.—Series of cross-sections of cotyledonary traces: *fig. 10*, just before joining vascular cylinder; *fig. 15*, after the several bundles have reunited in upper extremity of cotyledon; *px*, protoxylem. $\times 850$.

PLATE XXVII

FIGS. 16-21.—Series of cross-sections of leaf traces: *fig. 16*, just before joining vascular cylinder; *figs. 17-20*, at considerable intervals above each other; *fig. 21*, younger trace; shaded portion protophloem. $\times 850$.

PLATE XXVIII

FIG. 22.—Cross-section at low level of foliar trace of a three-year-old plant: *px*, protoxylem (endarch); *x*, xylem, of which probably the larger portion is centrifugal metaxylem and only a small portion is secondary xylem; *ph*, phloem; *pph*, protophloem. $\times 425$.

FIG. 23.—Section from the same strand as *fig. 22*, but considerably higher up; *px*, protoxylem (mesarch). $\times 425$.

FIG. 24.—Section of bundle from old leaf of quite a large plant just below rachis: *px*, protoxylem (exarch); *mx*, metaxylem (centripetal); *tc*, thin-walled cells; *sx*, secondary xylem; *ph*, phloem; *pph*, protophloem. $\times 425$.

FIG. 25.—Cross-section of bundle in pinna: lettering as in *fig. 24*; note absence of secondary xylem. $\times 425$.

FIG. 26.—Median longitudinal section of very young leaf trace, showing shape and arrangement of cells; shaded portion, earliest procambium. $\times 1200$.

FIG. 27.—Cross-section of the same bundle as in *fig. 26*.

PLATE XXIX

FIG. 28.—Median longitudinal section of embryo at right angles to inner faces of cotyledons. $\times 11$.

FIG. 29.—Median longitudinal section similar to *fig. 28*: *ct*, cotyledonary traces; *cp*, caplike tissue; *s*, suspensor. $\times 30$.

FIG. 30.—Cross-section of young seedling at region of union of cotyledonary trace with vascular cylinder: *ct*, cotyledonary traces belonging to one cotyledon. $\times 16$.

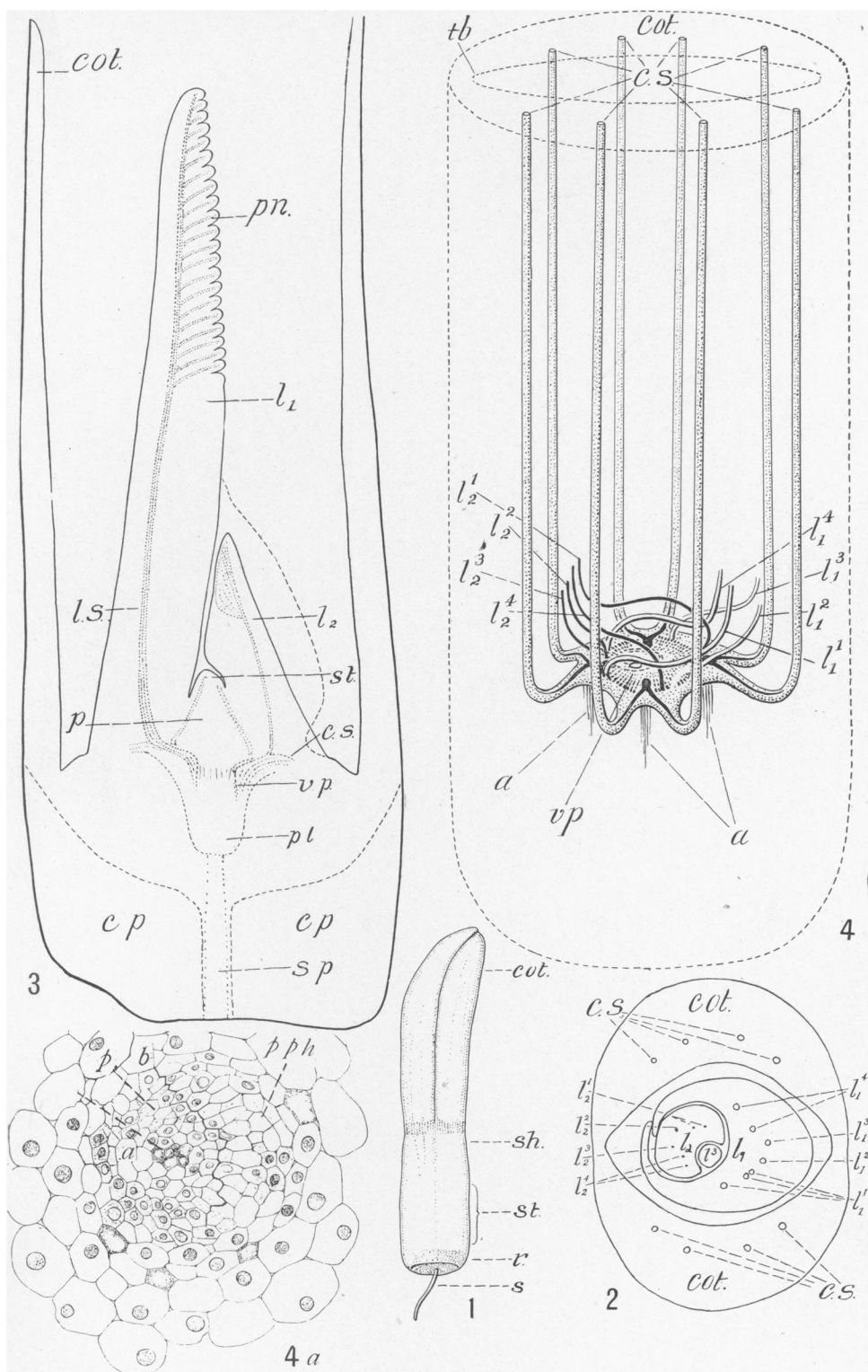
FIG. 31.—Section of same further up to show girdling: L_2^1 , outer long girdle; L_2^4 , outer shorter girdle; L_2^2 , L_2^3 , inner girdles; all of second leaf. $\times 16$.

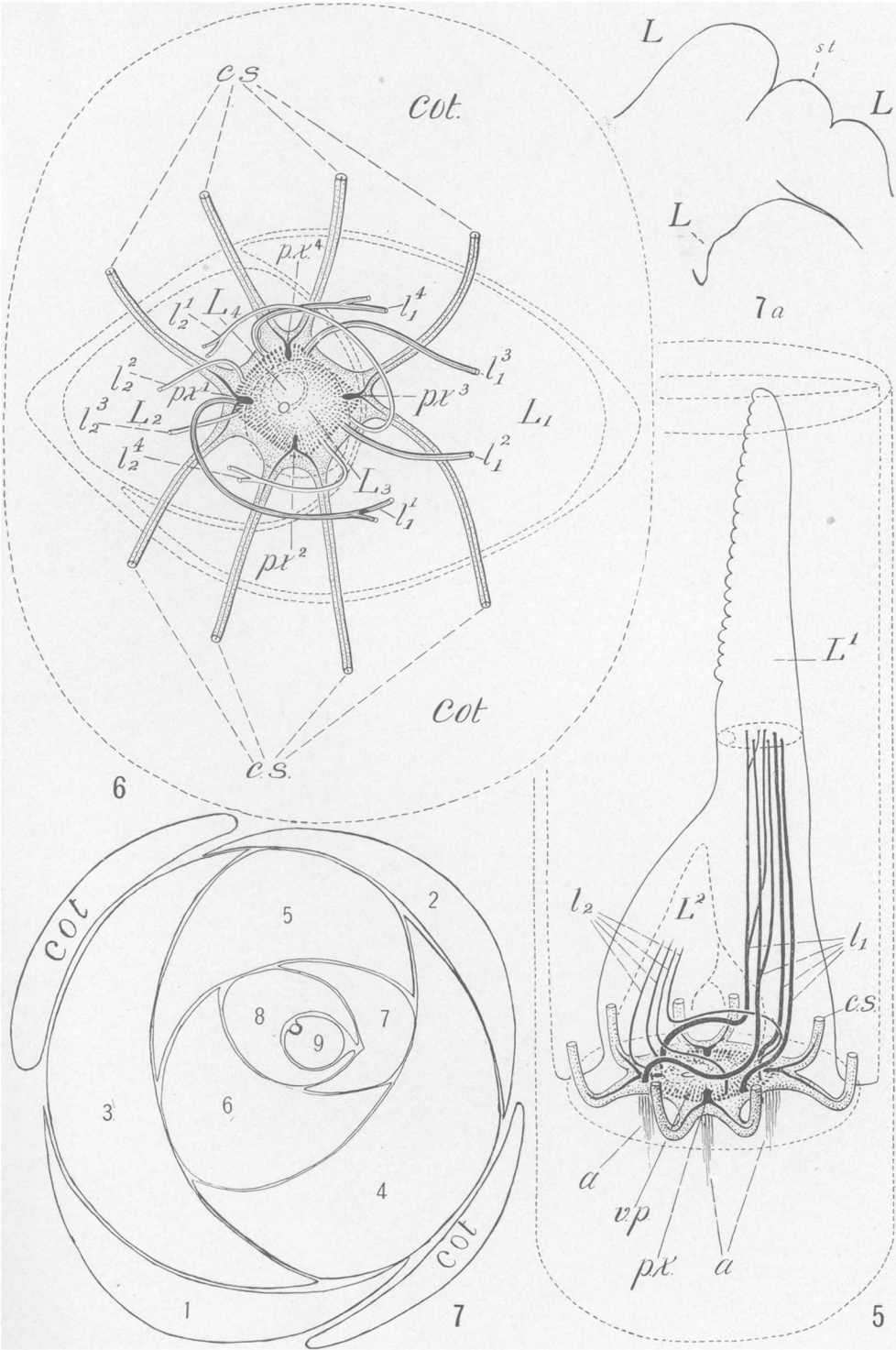
FIGS. 32, 33.—Cross-sections of embryo at region of vascular plate (*vp*); *px*, protoxylem; *x*, xylem; *ph*, phloem; union of cotyledonary traces two by two, and union of resultants with vascular plate at protoxylem groups. $\times 30$.

FIG. 34.—Median longitudinal section of seedling three years old: *pc*, procambium; *lg*, girdle; *lt*, trace; *lg*, leaf gap. $\times 30$.

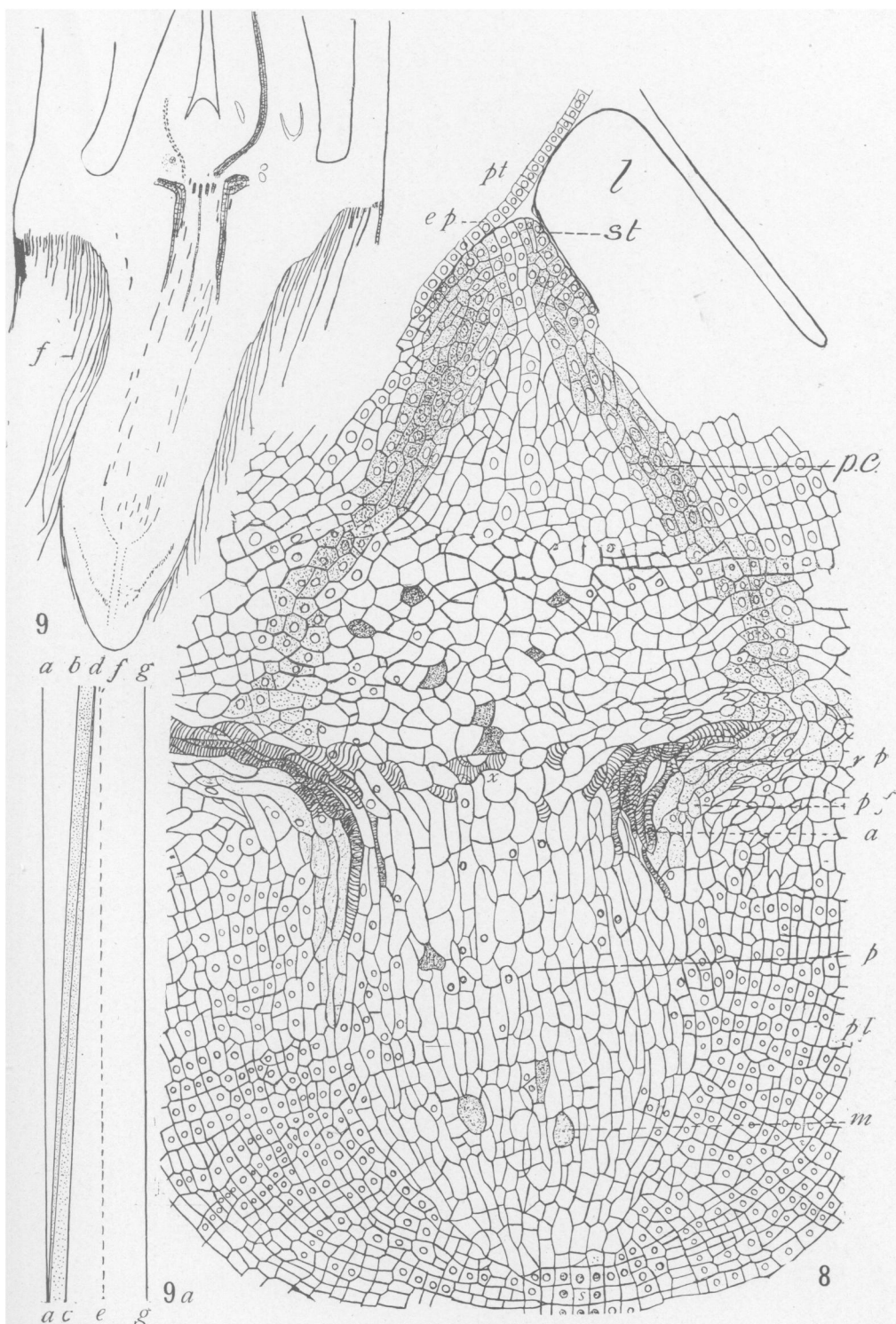
FIG. 35.—Cross-section of embryo to show early appearance of girdle; *pc*, procambium; lg^1 , girdle of very young leaf; lg^2 , girdle of next older leaf. $\times 30$.

The black spots in all the photographs are mucilage cavities or cells.

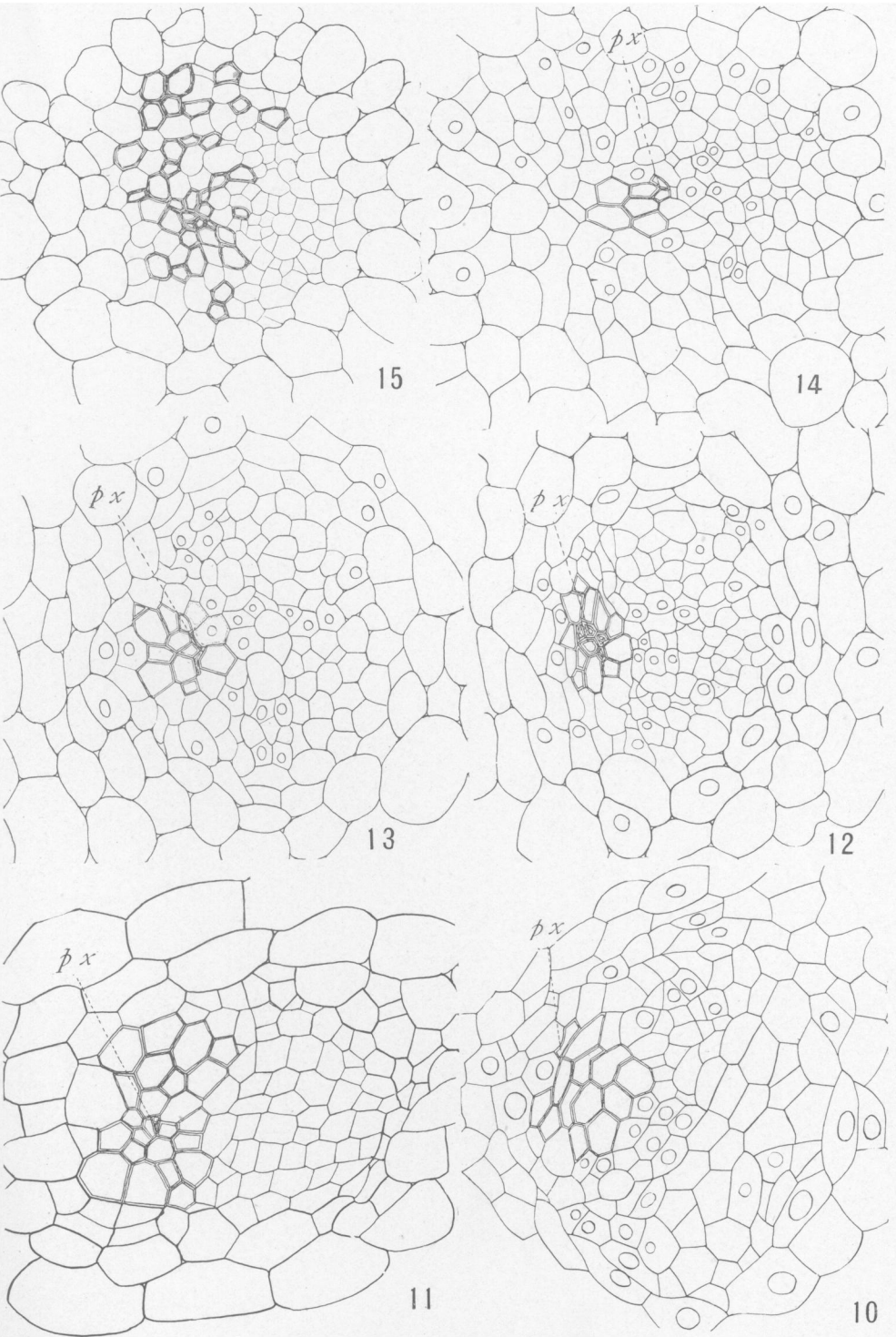




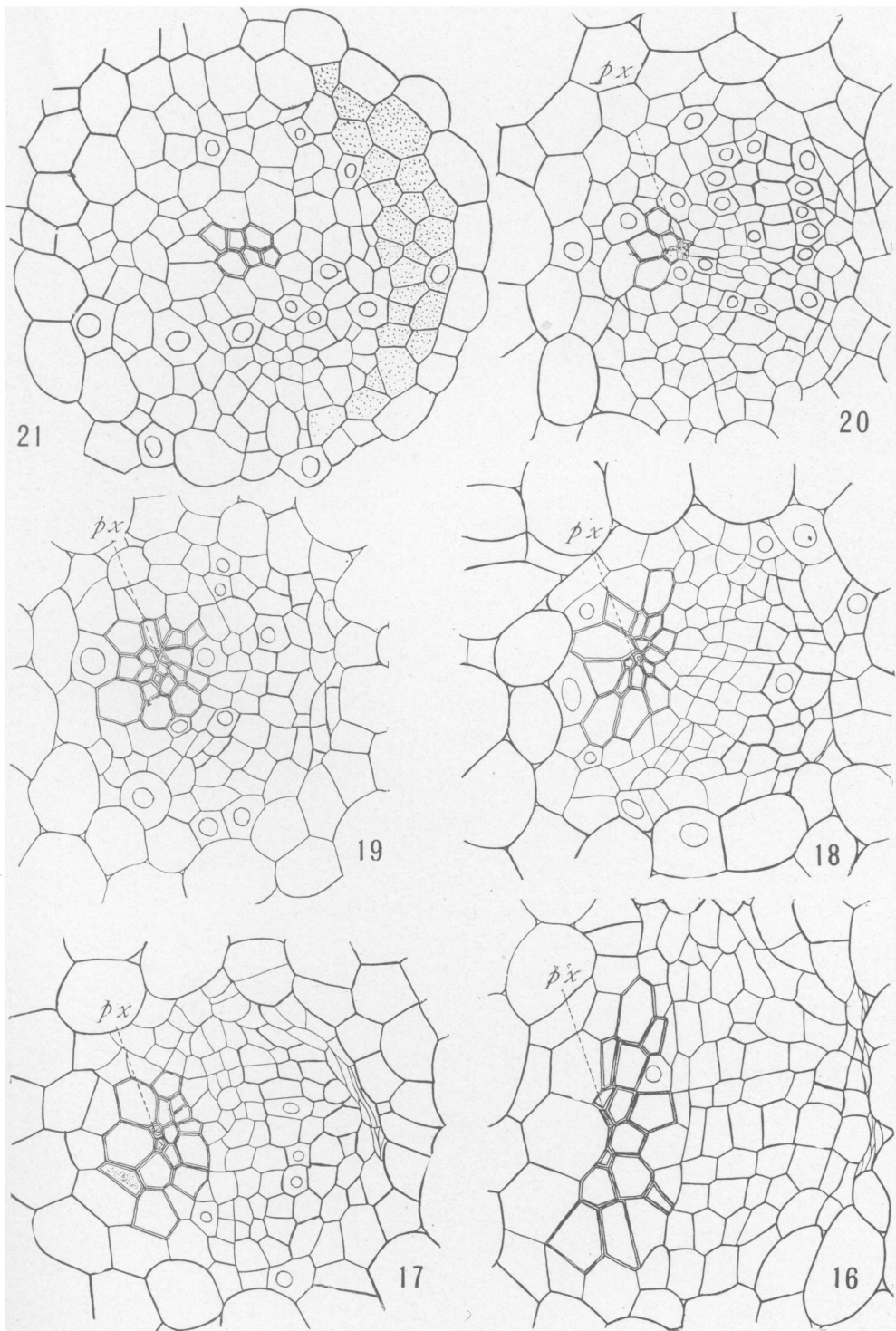
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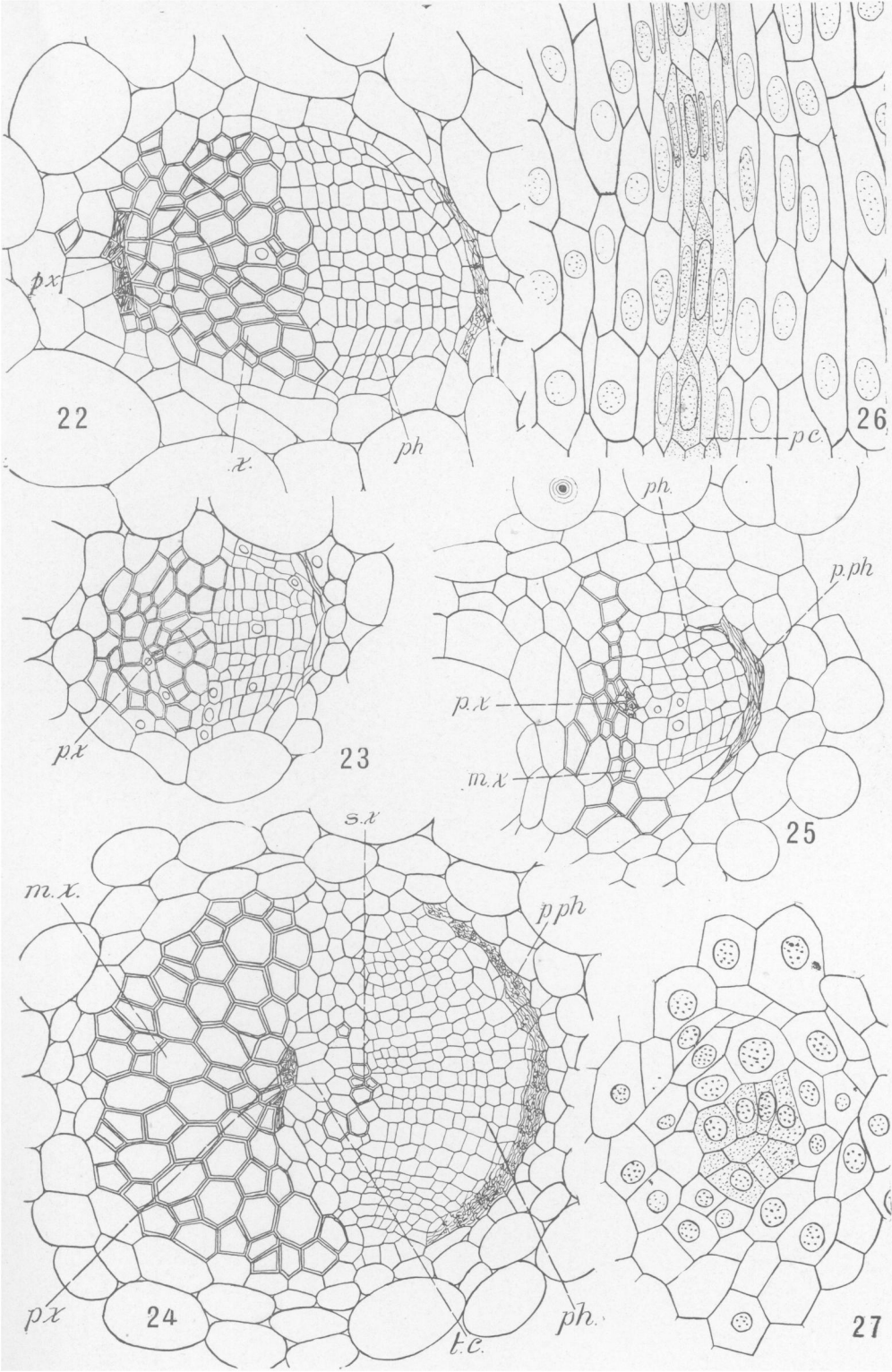
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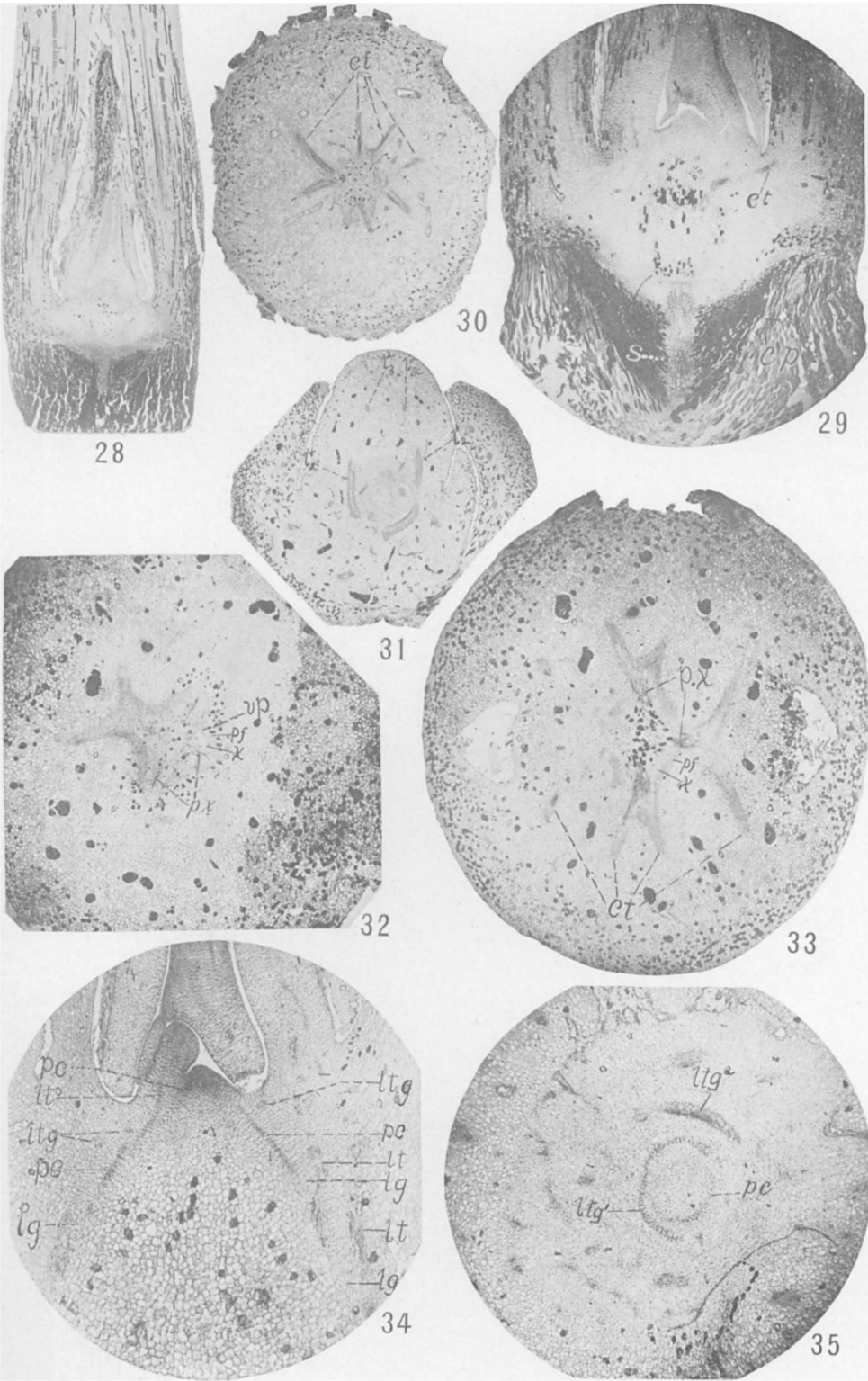
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